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Arbuscular mycorrhizal fungi enhance tolerance of vinca to high alkalinity in irrigation water

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Abstract

This study was conducted in order to determine if inoculation with arbuscular mycorrhizal fungi (AMF) would enhance the tolerance of vinca plants to high levels of alkalinity, induced by $KHCO_3$, in irrigation water. AMF-inoculated and non-inoculated plants were irrigated with water containing varying HCO_3^- concentrations: 0, 2.5, 5, 7.5, 10, and 15 mM. Increased HCO_3^- concentration inhibited plant growth, specifically at concentrations $\geq$7.5 mM. Leaves were more affected by high HCO_3^- concentration than other plant parts. In non-inoculated plants, a visual evaluation of quality demonstrated that acceptable quality was produced when irrigation water contained $\leq$2.5 mM HCO_3^-, but AMF-inoculated plants of good quality were produced when irrigation water contained $\leq$7.5 mM HCO_3^-. In general, AMF alleviated HCO_3^- stress, as indicated by greater plant growth and ranking of quality. However, AMF-inoculated plants irrigated with 0 mM HCO_3^- exhibited reduced growth when compared to non-inoculated plants. Bicarbonate did not affect leaf Fe concentration, indicating that vinca may be a Fe efficient plant. Plants inoculated with AMF exhibited an enhanced leaf P concentration and content, which was related to an increase in the activity of the soluble alkaline phosphatase. Plants inoculated with AMF exhibited increased leaf concentration and content of Mn, Zn, Cu, B, and Mo, and increased antioxidant activity under high concentrations of HCO_3^-. In conclusion, the tolerance of vinca to alkalinity in irrigation water can be enhanced by AMF inoculation, thus, allowing for irrigation with water of high alkalinity.

Keywords: Alkaline phosphatase; Antioxidant activity; Bicarbonate; Nitrate reductase; Photosynthesis rate; Plant quality

1. Introduction

Water availability and quality are issues of major concern worldwide. Water of good quality is limited especially in arid and semiarid regions of the world (Grieve et al., 2005); although in temperate regions some countries may face similar problems (Sonneveld and Voogt, 2003). Environmental concerns and intensifying competition for municipal and industrial water will further reduce the availability of good quality water resources for agricultural and horticultural production (Rosegrant and Ringler, 1998), specifically irrigation of landscape areas. The use of water of low quality for irrigation of ornamental and landscape species will assist in the conservation of water of better quality for other purposes.

Alkalinity and salinity of water are of concern because of their deleterious effect on plant nutrition and growth. Salinity is caused by an excessive accumulation of ions, principally Na^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, and HCO_3^-, which may have toxic effects on plant physiology and/or affect plant growth through osmotic effects. Alkalinity is caused by HCO_3^- and CO_3^{2-}, the major alkalis that impart a buffer capacity to water and cause an increase in solution pH, leading to the formation of insoluble forms of P and micronutrients. Chlorosis in young leaves is frequently observed on plants irrigated with water of high alkalinity, which may also inhibit growth of sensitive plants through reduced root growth and/or nutrient uptake and utilization (Alhendawi et al., 1997). These effects may represent a significant reduction in the marketability, aesthetic value, and/or performance of plants in the landscape. Greenhouse growers and the public are reluctant to use water of low quality for irrigation because they consider ornamental species to be highly sensitive to salinity and alkalinity. However,

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studies have demonstrated that some ornamental plants can be grown at high levels of salinity (Shillo et al., 2002; Grieve et al., 2005) or alkalinity (Valdez-Aguilar and Reed, 2007). Cartmill et al. (2007) demonstrated the feasibility of enhancing the tolerance of *Rosa multiflora*, an alkalinity-sensitive species, when plants were inoculated with arbuscular mycorrhizal fungi (AMF). It has been reported that AMF enhance plant nutrient acquisition (Clark and Zeto, 2000), water relations (Augé, 2001), and alleviate cultural and environmental stresses (Jeffries et al., 2003) through greater effective root area and penetration of the substrate, and the activation and excretion of various enzymes by AMF roots and/or hyphae (Marschner, 1995; Smith and Read, 1997).

The objective of this study was to determine if inoculation of vinca (*Catharanthus roseus* (L.) G. Don) plants with AMF would affect plant growth, nutrient status, photosynthetic rate, and the phosphatase, nitrate reductase, and antioxidant activities when exposed to increasing levels of alkalinity in irrigation water.

2. Materials and methods

2.1. Cultural conditions

This study was conducted under glasshouse conditions at Texas A&M University, College Station, Texas, from 6 July 2005 to 25 August 2005. Temperature and relative humidity were measured and recorded hourly for the duration of the experiment. Average day/night temperature and relative humidity were 30.9 ± 0.2 °C/25.5 ± 0.1 °C, and 69.5 ± 0.6%/82.9 ± 0.4%, respectively. Average daily photosynthetically active radiation (PAR) and PAR at approximately solar noon were 302.7 ± 10.2 and 519.5 ± 39.2 μmol m^{-2} s^{-1}, respectively. Vinca plugs were transplanted into 0.65 L green plastic containers. The container substrate was a peat, perlite, and vermiculite mix (2:1:1 by volume), which was amended with 8 kg m^{-3} controlled release fertilizer (15–3.9–9.9), 3.5 kg m^{-3} dolomitic limestone, 1.75 kg m^{-3} gypsum, and 0.9 kg m^{-3} micronutrients (Micromax). The peat was previously steam pasteurized with aerated steam on 2 consecutive days for 3 h at 80 °C.

2.2. Arbuscular mycorrhizal inoculation

Half the plugs were inoculated at planting with approximately 700 spores of a mixed *Glomus* species isolate (ZAC-19): *Glomus albidum* Walker & Rhodes, *Glomus claroideum* Schenck & Smith, and *Glomus diaphanum* Morton & Walker (Chamizo et al., 1998). The ZAC-19 inoculum was applied directly to the dibble hole at transplanting (approximately 10 g per pot) and included hyphae and colonized root segments of *Carica papaya* L. used for isolate multiplication. The remaining plugs were non-inoculated (non-AMF).

2.3. Bicarbonate application

Ten days after transplanting, plants were irrigated with approximately 100 mL of HCO_3^- ($KHCO_3$) solution, at 0, 2.5, 5, 7.5, 10, and 15 mM HCO_3^- every 4 days for the duration of the study. Average irrigation water pH was 7.10 ± 0.11, 8.12 ± 0.03, 8.31 ± 0.02, 8.40 ± 0.02, 8.42 ± 0.03, and 8.46 ± 0.04, and EC (dS m^{-1}) was 0.02 ± 0.00, 0.30 ± 0.01, 0.58 ± 0.01, 0.84 ± 0.02, 1.14 ± 0.04, and 1.68 ± 0.07, respectively. Plants were irrigated to achieve approximately 20% leachate fraction by volume. At experiment termination, the growing substrate of each pot was air dried, and subsamples were removed to determine EC and pH by the 1:2 dilution method (Warncke and Krauskopf, 1983).

2.4. Assessment of plant growth and photosynthetic rate

Visual evaluation of plant quality was taken at harvest. Evaluation criteria included: 1 = dead plant; 2 = most leaves necrotic and/or senescent; 3 = some chlorosis and necrosis and/or senescent; 4 = initial leaf chlorosis; 5 = green, healthy plant ($n = 4$). Net photosynthesis rate was determined with a portable photosynthesis system (LI-6400, LI-COR, Lincoln, Nebr.), with red/blue LED light source at PAR levels of 500 μmol m^{-2} s^{-1}, and CO_2 concentration of 360 ppm from fully turgid, expanded, uniform leaves ($n = 4$). Photosynthesis on a whole plant basis was calculated {Photosynthesis μmol $plant^{-1}$ s^{-1} = [(photosynthetic rate in μmol m^{-2} s^{-1}/10000) × leaf area in cm^2]}.

Final growth measurements were recorded at harvest ($n = 4$). Tissue samples were dried for 7 days at 70 °C and flower, leaf, stem, root, and total dry mass (DM) were recorded. Leaf area ratio {LAR = [leaf area (cm^2)]/plant DM (g^{-1})]} and specific leaf area {SLA = [leaf area (cm^2)]/[leaf DM (g^{-1})]} were calculated. The mycorrhizal inoculation effect (MIE) was calculated by the formula MIE (%) = (total DM of AMF plant − total DM of non-AMF plant)/(total DM of non-AMF plant)$^{-1}$ × 100 (Plenchette et al., 1983; Sylvia, 1994).

2.5. Leaf nutrient analysis

Physiologically mature leaves from three randomly selected plants per treatment were collected at harvest ($n = 3$) and ground to pass a 40-mesh screen. Tissue analysis of P, K, Mg, Ca, S, Na, Fe, Mn, Zn, Cu, Al, B, and Mo, was conducted on an inductively coupled plasma atomic emission spectrophotometer (Model Optima 4300V ICP-OES, PerkinElmer Life and Analytical Sciences, Inc., Boston, Mass.) on nitric acid/hydrogen peroxide digestions of leaf samples, while leaf N concentration was determined with Kjedlahl digestion. Results are reported as leaf nutrient concentration and leaf nutrient content.

2.6. Leaf chlorophyll concentration, antioxidant activity, nitrate reductase activity, and root phosphatase activity

Leaf chlorophyll concentration and antioxidant activity were determined by extraction with acetone (Harborne, 1998) and by extraction of soluble antioxidants (Re et al., 1999), respectively. Leaf nitrate reductase activity and root alkaline phosphatase (ALP) enzymatic activity (soluble and extractable) were

determined based on the formation of NO_2 (Foyer et al., 1998) and based on the hydrolysis of *p*-nitrophenyl phosphate (*p*-NPP) substrate to yield *p*-nitrophenol (*p*-NP) and inorganic phosphatase (Eivazi and Tabatabai, 1977; Tabatabai and Bremner, 1969), respectively. These analyses were performed at harvest ($n = 4$).

2.7. Arbuscular mycorrhizal development

For AMF colonization analysis, 1-cm root segments from four randomly selected plants per treatment were sampled at harvest and pooled to assess colonization percentage through clearing with KOH and staining of root samples with trypan blue (Philips and Hayman, 1970). Twenty 1-cm stained root pieces were placed on each slide and three observations (the top, the middle, and the bottom) per 1-cm root piece were made with a microscope at 40×. The presence of arbuscules, vesicles, and hyphae was determined (Biermann and Linderman, 1981). There were four slides per treatment ($n = 240$ observations per treatment from 80 1-cm root pieces).

2.8. Statistical design and analysis

The experiment was a 2×6 factorial in a completely randomized design with two AMF levels (AMF and non-AMF) and six levels of HCO_3^-: 0, 2.5, 5, 7.5, 10, and 15 mM HCO_3^-. There was one plant per container, with each container as a single replicate. Number of replicates varied depending on the growth attributes and enzymatic activities analyzed and were mentioned previuosly. Data were analyzed using analysis of variance (ANOVA) and Tukey's multiple comparison test ($P < 0.05$). Linear and quadratic effects within each AMF level were also estimated (SAS Institute Inc., 2000).

3. Results

3.1. Arbuscular mycorrhizal development

At experiment termination, an average total AMF colonization of 55% was observed in AMF-inoculated plants (Table 1). In non-AMF plants no colonization was observed (data not shown). Increasing HCO_3^- concentration did not significantly affect total colonization or vesicle formation, although the percentage of arbuscules was significantly decreased when plants were irrigated with water containing ≥10 mM HCO_3^-, compared to plants irrigated with water containing 2.5 mM HCO_3^-.

3.2. Substrate pH and EC

Increasing HCO_3^- concentration was associated with a significant increase in substrate pH and substrate EC (Table 2). AMF inoculation had a non-significant effect on substrate pH and substrate EC (Table 2). However, substrate pH was significantly affected by the HCO_3^- × AMF interaction, primarily because at low concentrations of HCO_3^- it was lower in AMF-inoculated plants than in non-AMF plants, but higher when HCO_3^- was ≥10 mM.

Table 1
Effect of bicarbonate (HCO_3^-) on the percent formation of arbuscules, vesicles, and colonization in root cortical cells of arbuscular mycorrhizal fungi (AMF)-inoculated vinca *Catharanthus roseus* 'Pacifica White' plants at experiment termination

Bicarbonate (mM)	Arbuscules	Vesicles	Total colonization
0	18.0	34.5	57.9
2.5	26.5	37.3	61.7
5	22.3	23.3	50.9
7.5	22.2	21.7	56.8
10	9.5	31.5	57.1
15	7.5	28.7	48.3
$LSD_{0.05}$[a]	10.9	18.0	24.0
Trend[b]			
Linear	<0.0001	0.1677	0.2251
Quadratic	0.0011	0.0821	0.7055
ANOVA[c]			
HCO_3^-	***	NS	NS

[a] Least significant difference according to Tukey's multiple comparison test at $P < 0.05$.
[b] P values.
[c] NS and ***, non-significant and significant $P < 0.001$, respectively.

3.3. Plant growth attributes

Bicarbonate caused a significant reduction in flower, leaf, stem, and total DM, but root DM was unaffected (Table 3). AMF inoculation did not have a significant effect on these plant growth attributes; however, AMF interacted significantly with HCO_3^- to affect flower, leaf, and total DM. The significant interaction was mainly due to the linear decrease in dry mass in non-AMF plants, while in AMF-inoculated plants the response was quadratic. The quadratic effect in AMF plants was because

Table 2
Effect of bicarbonate (HCO_3^-) and arbuscular mycorrhizal fungi (AMF) on substrate pH and electrical conductivity (EC) at experiment termination

HCO_3^- (mM)	pH		EC (dS cm^{-1})	
	Non-AMF	AMF	Non-AMF	AMF
0	5.7	5.6	1.2	1.1
2.5	6.2	6.0	1.6	1.8
5.0	6.7	6.8	1.7	1.8
7.5	7.4	7.3	1.9	1.8
10	7.6	7.8	1.9	1.9
15	7.9	8.1	1.8	2.1
$LSD_{0.05}$[a]	0.1	0.1	0.6	0.3
Trend[b]				
Linear	<0.001	<0.001	<0.001	<0.001
Quadratic	<0.001	<0.001	0.018	0.001
ANOVA[c]				
HCO_3^-	***		***	
AMF	NS		NS	
Interaction	***		NS	

[a] Least significant difference according to Tukey's multiple comparison test at $P < 0.05$.
[b] P values.
[c] NS and ***, non-significant and significant $P < 0.001$, respectively.

Table 3
Effect of bicarbonate (HCO_3^-) and arbuscular mycorrhizal fungi (AMF) on dry mass (DM) (in grams) and the mycorrhizal inoculation effect (MIE), of vinca *Catharanthus roseus* 'Pacifica White' at experiment termination

HCO_3^- (mM)	Flower DM		Leaf DM		Stem DM		Root DM		Total DM		MIE[a] (%)
	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	
0.0	1.4	0.9	4.5	3.5	2.1	1.6	0.7	0.6	8.7	6.4	−26.4
2.5	1.7	0.9	4.6	3.8	2.5	1.9	0.7	0.6	9.5	7.2	−24.2
5.0	1.2	1.1	3.6	3.8	2.1	2.1	0.7	0.7	7.6	7.7	1.3
7.5	0.7	1.1	2.7	3.6	1.8	2.0	0.8	0.7	5.9	7.3	23.7
10	0.4	0.7	2.7	2.9	1.8	1.8	0.7	0.7	5.6	6.0	7.1
15	0.1	0.1	1.2	1.1	1.6	1.4	0.7	0.6	3.5	3.2	−8.6
$LSD_{0.05}$[b]	0.7	0.3	1.9	1.1	1.1	0.7	0.2	0.2	3.4	1.4	
Trend[c]											
Linear	<0.001	<0.001	<0.001	<0.001	0.023	0.349	0.879	0.355	<0.001	<0.001	
Quadratic	0.103	<0.001	0.314	<0.001	0.593	0.003	0.190	0.003	0.248	<0.001	
ANOVA[d]											
HCO_3^-	***		***		*		NS		***		
AMF	NS		NS		NS		NS		NS		
Interaction	***		*		NS		NS		*		

[a] Mycorrhizal inoculation effect [MIE (%) = (total DM of AMF plant − total DM of non-AMF plant)/(total DM of non-AMF plant)$^{-1}$ × 100].
[b] Least significant difference according to Tukey's multiple comparison test at $P < 0.05$.
[c] P values.
[d] NS, * and ***, non-significant and significant $P < 0.05$, 0.001, respectively.

dry mass was lower in plants irrigated with 0 mM HCO_3^-, even lower than that of non-AMF plants, but plants irrigated with 2.5, 5, and 7.5 mM HCO_3^- water exhibited an increase in dry mass. Higher than 10 mM HCO_3^- concentrations caused the plant growth to decrease. Leaf number and leaf area were also significantly affected by the HCO_3^- × AMF interaction (Table 4), with similar trends to those reported for dry mass.

The SLA and LAR were affected significantly by AMF inoculation (Table 4). Averaged over all concentrations of HCO_3^-, AMF plants exhibited a significant 22% and 27% higher SLA and LAR, respectively, compared to non-AMF plants.

Visual evaluation of plant quality was significantly affected by increasing HCO_3^- concentration and AMF inoculation (Table 5). Non-AMF plants irrigated with water containing 5 mM HCO_3^- exhibited a 27% decrease in the visual aesthetic value when compared to non-AMF control plants. Arbuscular mycorrhizal plants irrigated with water containing 7.5 mM HCO_3^- exhibited a similar decrease (35%) in aesthetic value as the non-AMF plants at 2.5 mM HCO_3^-. The significant HCO_3^- × AMF interaction suggests that in AMF-inoculated plants the decrease was less marked when HCO_3^- concentration increased (quadratic trend), compared to non-AMF plants (linear trend).

Table 4
Effect of bicarbonate (HCO_3^-) and arbuscular mycorrhizal fungi (AMF) on leaf growth of vinca *Catharanthus roseus* 'Pacifica White'

HCO_3^- (mM)	Leaf number		Leaf area (cm^2)		SLA[a] ($cm^2\ g^{-1}$)		LAR[b] ($cm^2\ g^{-1}$)	
	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF
0.0	292.5	257.5	1338.3	1278.4	296.1	363.5	154.0	197.3
2.5	308.0	261.8	1492.8	1319.1	324.9	346.9	158.5	182.7
5.0	254.0	283.0	1213.5	1483.7	331.7	386.0	157.1	190.5
7.5	159.5	279.5	826.2	1383.6	311.0	389.0	138.7	189.5
10	154.8	263.3	753.6	1093.2	273.1	382.0	129.5	180.4
15	79.5	110.8	349.4	393.0	290.1	369.7	100.5	122.7
$LSD_{0.05}$[c]	117.5	83.8	657.0	503.3	65.2	57.1	37.3	55.5
Trend[d]								
Linear	<0.001	<0.001	<0.001	<0.001	0.107	0.207	<0.001	0.002
Quadratic	0.298	<0.001	0.159	<0.001	0.091	0.179	0.018	0.022
ANOVA[e]								
HCO_3^-	***		***		NS		***	
AMF	*		*		***		***	
Interaction	***		***		NS		NS	

[a] Specific leaf area.
[b] Leaf area ratio.
[c] Least significant difference according to Tukey's multiple comparison test at $P < 0.05$.
[d] P values.
[e] NS, * and ***, non-significant and significant $P < 0.05$, 0.001, respectively.

Table 5
Effect of bicarbonate (HCO_3^-) and arbuscular mycorrhizal fungi (AMF) on visual aesthetic value and some physiological responses of vinca *Catharanthus roseus* 'Pacifica White'

HCO_3^- (mM)	Visual evaluation[a]		Total chlorophyll μg cm^{-2}		Photosynthesis rate μmol CO_2 m^{-2} s^{-1}		Nitrate reductase μmol NO_2 g^{-1}		Antioxidant activity mmol Trolox g^{-1}		Alkaline phosphatase μmol *p*-NP g^{-1} root fresh mass h^{-1}			
											Extractable		Soluble	
	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF
0.0	4.33	4.33	31.7	31.3	11.3	18.3	117.4	112.2	8.67	7.92	8.70	7.0	13.6	14.0
2.5	3.83	4.17	33.9	28.9	19.3	13.2	113.0	92.8	7.26	6.37	8.56	8.2	13.2	15.5
5.0	3.17	4.00	30.8	26.7	19.0	18.8	137.8	84.3	8.56	5.69	9.03	10.1	17.1	20.2
7.5	3.00	3.75	31.9	29.2	19.4	24.0	146.8	100.0	6.82	7.46	9.57	9.1	13.8	18.4
10	2.67	2.83	23.5	26.8	7.4	11.3	102.6	152.9	7.45	9.18	14.00	10.7	17.1	12.9
15	2.00	2.00	19.6	24.3	6.4	7.9	142.3	104.6	4.86	6.49	14.20	10.4	23.7	19.6
$LSD_{0.05}$[b]	0.31	0.23	8.05	6.00	13.7	16.7	76.7	67.1	2.66	3.18	5.1	4.3	6.1	8.4
Trend[c]														
Linear	<0.001	<0.001	<0.001	0.003	0.030	0.098	0.482	0.223	<0.001	0.614	<0.001	0.008	<0.001	0.250
Quadratic	0.433	<0.001	0.006	0.889	0.005	0.069	0.725	0.468	0.130	0.552	0.106	0.348	0.017	0.408
ANOVA[d]														
HCO_3^-	***		***		***		NS		**		***		***	
AMF	***		NS		NS		*		NS		*		NS	
Interaction	***		*		NS		*		**		NS		*	

[a] 1 = dead plant, 2 = most leaves necrotic/senescent, 3 = some chlorosis and necrosis/senescent, 4 = initial leaf chlorosis, 5 = healthy plant.
[b] Least significant difference according to Tukey's multiple comparison test at $P < 0.05$.
[c] *P* values.
[d] NS, *, ** and ***, non-significant and significant $P < 0.05$, 0.01, 0.001, respectively.

In general, most of the plant growth attributes were lower in AMF-inoculated plants, compared to non-AMF plants, when the concentration of HCO_3^- was between 0 and 2.5 mM (Tables 3 and 4); however, when HCO_3^- concentration was increased to 5–10 mM, AMF-inoculated plants exhibited higher growth, even surpassing that of non-AMF plants. This response can be numerically detected in the MIE (Table 3), which indicates that AMF colonization caused a decrease (negative MIE) in total DM at HCO_3^- concentrations ≤2.5 mM. Limited mycorrhizal effect on total DM was observed at 5 mM HCO_3^- (Table 3); however, at 7.5 and 10 mM HCO_3^-, AMF had a marked ameliorating effect (positive MIE) on total DM, and other plant growth attributes.

Table 6
Effect of bicarbonate (HCO_3^-) and arbuscular mycorrhizal fungi (AMF) on leaf macronutrients and Na concentration (g kg^{-1}) of vinca *Catharanthus roseus* 'Pacifica White'

HCO_3^- (mM)	N		P		K		Ca		Mg		S		Na	
	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF
0.0	16.5	16.9	1.3	1.7	21.0	31.0	8.2	12.1	4.0	6.6	1.5	2.0	0.7	0.7
2.5	17.7	16.9	1.3	1.8	29.1	41.8	6.5	9.6	3.8	5.6	1.6	2.0	0.9	0.8
5.0	17.8	19.6	1.3	1.8	37.2	47.4	4.9	5.7	2.8	3.6	1.5	2.1	0.7	0.7
7.5	20.4	19.8	1.3	1.7	49.0	53.9	4.3	4.6	2.6	3.0	1.6	2.0	0.7	0.6
10	18.4	21.3	1.1	1.8	44.7	69.2	3.8	4.5	2.6	2.7	1.4	2.3	0.7	0.6
15	18.9	20.7	1.0	1.7	70.2	86.9	4.5	5.2	2.7	2.8	1.5	1.8	0.8	0.7
$LSD_{0.05}$[a]	7.0	7.9	0.5	0.5	9.2	11.7	1.7	2.2	0.5	0.9	0.5	0.7	0.2	0.2
Trend[b]														
Linear	0.192	0.036	0.030	0.972	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.700	0.919	1.000	0.097
Quadratic	0.393	0.615	0.253	0.589	0.055	0.007	<0.001	<0.001	<0.001	<0.001	0.805	0.145	0.473	0.578
ANOVA[c]														
HCO_3^-	NS		NS		***		***		***		NS		*	
AMF	NS		***		***		***		***		***		*	
Interaction	NS		NS		**		***		***		NS		NS	

[a] Least significant difference according to Tukey's multiple comparison test at $P < 0.05$.
[b] *P* values.
[c] NS, *, ** and ***, non-significant and significant $P < 0.05$, 0.01, 0.001, respectively.

Table 7
Effect of bicarbonate (HCO_3^-) and arbuscular mycorrhizal fungi (AMF) on leaf micronutrient concentration (μg g^{-1}) of vinca *Catharanthus roseus* 'Pacifica White'

HCO_3^- (mM)	Fe		Mn		Zn		Cu		Al		B		Mo	
	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF
0.0	77.7	69.0	325.7	595.0	47.3	94.7	9.0	15.3	16.7	29.7	126.7	170.7	12.3	14.0
2.5	55.3	75.3	311.0	563.0	46.0	100.3	9.3	17.7	15.7	19.0	144.3	164.3	9.3	17.7
5.0	58.3	59.3	258.0	360.3	49.0	79.0	9.3	16.7	21.0	17.0	115.0	121.3	13.3	19.7
7.5	62.3	51.3	261.7	330.7	45.3	81.7	10.3	16.3	17.7	16.3	95.0	86.0	15.0	22.0
10	64.3	58.7	239.0	331.3	47.0	85.0	10.3	18.7	27.3	26.0	75.3	88.3	18.0	27.3
15	67.3	64.3	246.3	345.0	55.0	93.3	13.3	20.3	21.0	25.7	85.7	96.0	18.3	35.0
$LSD_{0.05}$[a]	39.8	17.0	90.1	120.7	10.7	37.9	2.4	4.6	13.1	18.1	44.9	40.5	8.3	9.2
Trend[b]														
Linear	0.773	0.019	0.002	<0.001	0.069	0.468	<0.001	0.005	0.039	0.992	<0.001	<0.001	0.002	<0.001
Quadratic	0.136	0.018	0.208	<0.001	0.069	0.151	0.014	0.284	0.718	0.015	0.982	0.007	0.442	0.084
ANOVA[c]														
HCO_3^-	NS		***		NS		***		*		***		***	
AMF	NS		***		***		***		NS		*		***	
Interaction	NS		***		NS		NS		NS		NS		*	

[a] Least significant difference according to Tukey's multiple comparison test at $P < 0.05$.
[b] P values.
[c] NS, * and ***, non-significant and significant $P < 0.05$, 0.001, respectively.

3.4. Leaf nutrient analysis

Increasing HCO_3^- concentration significantly increased leaf concentration of K (Table 6), Cu, and Mo (Table 7), but significantly decreased leaf Ca, Mg (Table 6), Mn, and B concentration (Table 7). AMF inoculation significantly increased leaf P, K, Ca, Mg (Table 6), Mn, Zn, Cu, B, and Mo concentration (Table 7), but leaf Na was significantly decreased (Table 6).

The significant HCO_3^- × AMF interaction for leaf Ca and Mg (Table 6) was primarily due to a higher Ca and Mg concentration at low HCO_3^- in AMF plants, when compared to non-AMF plants. At higher HCO_3^-, Ca and Mg concentration was comparable in AMF and non-AMF plants.

The HCO_3^- × AMF interaction was also significant for leaf K concentration (Table 6). Although the trend of increasing leaf K concentration as HCO_3^- increased was quadratic (Table 6), a linear trend best fit the response observed in AMF and non-AMF plants. The significant interaction was mainly due to the difference in the slope of the regression lines ($m = 3.1$ for non-AMF plants, and $m = 3.7$ for AMF plants).

The significant HCO_3^- × AMF interaction for leaf Mn concentration (Table 7) was primarily due to a less drastic

Table 8
Effect of bicarbonate (HCO_3^-) and arbuscular mycorrhizal fungi (AMF) on leaf macronutrients and Na content (g $plant^{-1}$) of vinca *Catharanthus roseus* 'Pacifica White'

HCO_3^- (mM)	N		P		K		Ca		Mg		S		Na	
	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF
0.0	72.0	66.5	5.79	6.42	90.1	118.4	35.0	46.3	17.0	25.3	6.52	7.64	2.99	2.70
2.5	79.7	62.6	5.80	6.53	129.0	155.2	29.9	35.7	17.1	20.9	7.16	7.55	3.86	2.86
5.0	61.5	78.4	4.49	7.19	127.1	188.5	16.9	22.6	9.7	14.5	5.20	8.50	2.36	2.78
7.5	58.4	68.6	3.85	5.79	142.4	185.7	12.3	15.9	7.5	10.5	4.60	7.04	2.06	2.05
10	43.4	57.3	2.63	4.73	102.9	185.7	8.7	12.1	5.7	7.2	3.26	6.19	1.59	1.62
15	22.9	23.0	1.25	1.90	84.6	95.3	5.4	5.7	3.3	3.1	1.84	2.01	0.95	0.73
$LSD_{0.05}$[a]	50.2	37.6	3.41	2.51	81.7	67.8	15.0	8.0	7.8	4.5	3.92	3.62	1.60	1.09
Trend[b]														
Linear	0.002	0.004	<0.001	<0.001	0.542	0.830	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic	0.209	0.003	0.335	<0.001	0.017	<0.001	0.131	0.003	0.526	0.133	0.324	0.002	0.289	0.008
ANOVA[c]														
HCO_3^-	***		***		***		***		***		***		***	
AMF	NS		***		***		**		***		***		NS	
Interaction	NS		NS		NS		NS		NS		NS		NS	

[a] Least significant difference according to Tukey's multiple comparison test at $P < 0.05$.
[b] P values.
[c] NS, ** and ***, non-significant and significant $P < 0.01$, 0.001, respectively.

Table 9
Effect of bicarbonate (HCO_3^-) and arbuscular mycorrhizal fungi (AMF) on leaf micronutrient content (μg plant^{-1}) of vinca *Catharanthus roseus* 'Pacifica White'

HCO_3^- (mM)	Fe		Mn		Zn		Cu		Al		B		Mo	
	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF	Non-AMF	AMF
0.0	331.1	267.0	1372	2273	202.5	359.8	38.9	59.0	72.5	114.5	547.4	656.1	52.7	54.0
2.5	248.3	280.6	1402	2085	212.0	371.2	41.4	65.6	70.0	70.9	637.7	609.8	43.7	65.8
5.0	203.0	236.3	891	1436	168.6	315.2	32.0	66.2	70.6	67.6	390.4	482.9	44.9	78.7
7.5	182.3	176.8	750	1146	131.5	287.3	30.1	56.8	51.0	55.3	273.4	295.5	41.9	75.9
10	151.2	156.4	530	895	106.6	227.8	23.7	50.1	65.2	68.3	164.4	239.6	39.7	72.7
15	81.1	70.9	291	374	65.6	103.1	16.1	22.6	25.2	28.5	101.0	101.7	22.1	39.0
$LSD_{0.05}$[a]	207.4	95.4	534	456	120.8	165.2	22.4	25.2	62.8	64.7	241.1	145.5	33.5	36.2
Trend[b]														
Linear	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.039	0.002	<0.001	<0.001	0.015	0.389
Quadratic	0.769	0.050	0.864	0.983	0.459	0.050	0.393	0.002	0.293	0.512	0.625	0.547	0.400	0.001
ANOVA[c]														
HCO_3^-	***		***		***		***		**		***		**	
AMF	NS		***		***		***		NS		NS		***	
Interaction	NS		*		NS		NS		NS		NS		NS	

[a] Least significant difference according to Tukey's multiple comparison test at $P < 0.05$.
[b] P values.
[c] NS, *, ** and ***, non-significant and significant $P < 0.05$, 0.01, 0.001, respectively.

decrease in leaf Mn concentration in AMF-inoculated plants, while for Mo the significant interaction was due to a more pronounced increase in leaf Mo when HCO_3^- concentration increased in AMF-inoculated plants.

In terms of content, all leaf nutrients were significantly affected by increasing HCO_3^- (Tables 8 and 9). AMF inoculation significantly increased leaf content of P, K, Ca, Mg, S (Table 8), Mn, Zn, Cu, and Mo (Table 9), regardless of the HCO_3^- level. The trend for the content of all the nutrients was primarily linear in non-AMF plants (except for K), and quadratic in AMF plants (except for Mg, Mn, Al, and B). The quadratic response in AMF plants was due to a higher leaf nutrient content under low HCO_3^- concentrations and because it was less affected by increasing HCO_3^- at ≤ 10 mM; however, 15 mM HCO_3^- caused a marked decrease in nutrient content. In non-AMF plants there was a linear decrease in leaf nutrient content as HCO_3^- concentration increased.

3.5. Total leaf chlorophyll concentration and photosynthesis rate

Total chlorophyll and photosynthetic rate were significantly affected by increasing concentrations of HCO_3^- (Table 5). Mycorrhizal inoculation did not have a significant effect, but it interacted significantly with HCO_3^- to affect total chlorophyll concentration. The significant $HCO_3^- \times$ AMF interaction is a result of the lower chlorophyll concentration in AMF plants, compared to non-AMF plants, when HCO_3^- concentration was $\leq$7.5 mM, but at higher concentrations, AMF plants had a higher chlorophyll concentration. Total chlorophyll concentration decreased linearly in AMF-inoculated plants, but in non-AMF plants the response was quadratic, suggesting that the decrease in chlorophyll was more pronounced in AMF plants when HCO_3^- concentration was $\leq$7.5 mM. This may be due to a dilution effect since leaf area of AMF plants was higher than that of non-AMF plants at HCO_3^- concentrations between 5 and 10 mM.

Photosynthetic rate showed a quadratic response to increasing HCO_3^- in non-AMF and AMF plants (Table 5). This was because at moderate levels of HCO_3^-, between 0 and 7.5 mM, photosynthetic rate was not significantly affected, but when HCO_3^- was $\geq$10 mM there was a steep decrease. On a whole plant basis (considering the total leaf area surface per plant), total plant photosynthesis was higher in AMF plants at HCO_3^- concentrations between 5 and 10 mM (Fig. 1). Total DM in AMF plants increased when photosynthesis rate

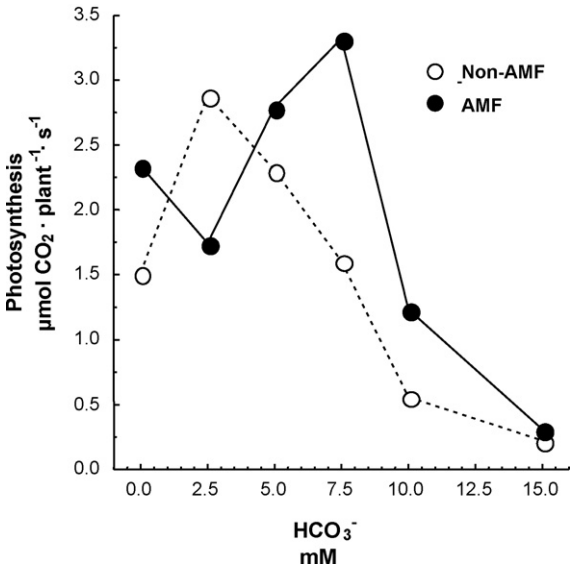

Fig. 1. Photosynthesis on a whole plant basis in arbuscular mycorrhizal fungi (AMF) (solid line, closed symbols) and non-AMF (dashed line, open symbols) vinca *Catharanthus roseus* 'Pacifica White' plants, treated with increasing concentrations of HCO_3^- in irrigation water.

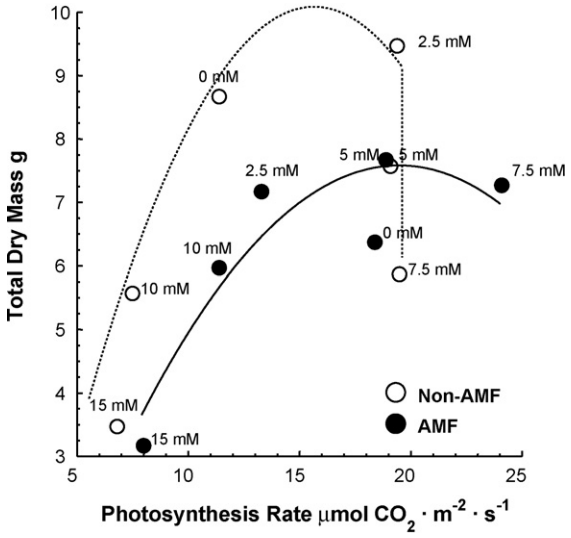

Fig. 2. Relationship between photosynthesis rate and total dry mass in non-arbuscular mycorrhizal fungi (AMF) (dashed line, open symbols) and AMF-inoculated (solid line, closed symbols) vinca *Catharanthus roseus* 'Pacifica White' plants. Legends near the closed or open symbols indicate the concentration of HCO_3^-.

increased from 7.9 to 13.2 μmol CO_2 m^{-2} s^{-1} (Fig. 2); however, higher photosynthetic rates were not associated with higher dry mass accumulation, probably because those higher rates were combined with high HCO_3^- concentrations. Non-AMF inoculated plants exhibited a similar trend to AMF plants; however, at comparable photosynthetic rates, non-AMF plants produced more dry mass than AMF plants. In non-AMF plants, photosynthetic rate also was associated with an increase in total DM when it increased from 6.4 to 19.3 μmol CO_2 m^{-2} s^{-1}, but at higher photosynthetic rates there was not increase in total DM accumulation, probably due to the increase in HCO_3^- concentration from 2.5 to 7.5 mM.

3.6. Leaf nitrate reductase activity, antioxidant activity, and root phosphatase activity

Leaf nitrate reductase activity was significantly lower in AMF-inoculated plants compared to non-AMF plants (Table 5). Increasing concentration of HCO_3^- in irrigation water did not affect the activity of the nitrate reductase; however, the HCO_3^- × AMF resulted significant.

AMF did not have a significant effect on leaf antioxidant activity (Table 5) but it interacted significantly with HCO_3^-. The interaction was a result of leaf antioxidant activity not being affected in AMF-inoculated plants when HCO_3^- was increased, while in non-AMF plants there was a linear decrease in the activity with increasing alkalinity in irrigation water.

Extractable and soluble alkaline phosphatase activities were significantly increased with high HCO_3^- concentrations (Table 5). In non-AMF plants, the increase in the soluble and extractable phosphatase activity was linear or quadratic and they were significantly higher when HCO_3^- concentrations were ≥10 mM, when compared to plants treated with 0 mM HCO_3^-. In AMF-inoculated plants, extractable alkaline phosphatase activity was significantly lower than in non-AMF plants. Bicarbonate and AMF inoculation interacted significantly to affect the activity of the soluble alkaline phosphatase. This was because soluble alkaline phosphatase activity was higher in AMF-inoculated plants when HCO_3^- was between 0 and 7.5 mM, but when HCO_3^- concentration was ≥10 mM, the enzymatic activity was lower than that of non-AMF plants.

4. Discussion

Increasing alkalinity of irrigation water inhibited growth of vinca plants, specifically at concentrations ≥10 mM HCO_3^-. This is in agreement with previous reports by Valdez-Aguilar and Reed (2007). Most of the plant growth attributes showed a quadratic trend when plants were inoculated with AMF, while non-AMF plants exhibited a decreasing linear trend. The quadratic trend was mainly due to the lack of effect of increasing HCO_3^- from 0 mM to 7.5 or 10 mM in AMF plants, while in non-AMF plants, growth was decreased at lower HCO_3^- levels. This implies that AMF inoculation assisted vinca plants to alleviate alkalinity stress and that non-inoculated plants were more sensitive to alkalinity. Inoculated plants of good quality were obtained when water contained up to 7.5 mM HCO_3^- but comparable quality was obtained when water contained 2.5 mM HCO_3^- in non-inoculated plants, suggesting that AMF inoculation allows for the production of vinca plants of good quality even when irrigated with water of high alkalinity.

Despite the enhanced tolerance to alkalinity, AMF-inoculated plants showed decreased growth and dry mass accumulation, compared to non-AMF plants, when irrigated with water containing 0–2.5 mM HCO_3^-. This may be due to the carbon required to maintain the respiration by the fungi under non-limiting environmental conditions (Bryla and Eissenstat, 2005). The use of carbon by the fungi was reflected by a decrease in total DM in AMF plants, which accumulated less dry mass even at photosynthetic rates comparable to those of non-AMF plants. The decreased growth in AMF plants can be numerically detected in the MIE, which indicates that AMF inoculation caused a decrease in total DM at HCO_3^- concentrations ≤2.5 mM. However, at 7.5 and 10 mM HCO_3^-, AMF enhanced growth, surpassing that of non-AMF plants. This enhanced growth at higher HCO_3^- in AMF plants was supported by a higher leaf area, which allowed increasing photosynthesis on a whole plant basis and provided the carbon required to maintain the mycorrhizal association.

High HCO_3^--induced alkalinity has been reported to cause leaf chlorosis, which is usually associated with a Fe deficiency due to a reduction in Fe availability at high pH (Alhendawi et al., 1997; Römheld, 2000). Bicarbonate can also cause an internal precipitation of Fe in plant tissues, rendering Fe inactive in the roots due to tissue alkalization (Römheld, 2000). In the present study we did not detect an effect of HCO_3^- or AMF on leaf Fe concentration; however, increasing HCO_3^- in irrigation water was associated with a decrease of leaf Fe in

terms of nutrient content. Leaf Fe content was significantly decreased only when HCO_3^- concentration was $\geq$7.5 mM in AMF and non-AMF plants. This suggests that vinca may be Fe efficient, since this concentration is enough to induce Fe deficiency in many plant species. This is also supported by the fact that a high concentration of HCO_3^- (7.5 mM) was required to induce a significant decrease in total chlorophyll concentration, whose synthesis requires Fe.

It has been reported that AMF may improve Fe nutrition by producing Fe chelators (Cress et al., 1986) that enhance Fe uptake. In our study, AMF inoculation was not associated with changes in leaf Fe concentration or content, but in a previous report we found contrasting results in *R. multiflora*, since increasing HCO_3^- concentration from 0 to 10 mM was associated with a decrease of up to 48% in leaf Fe concentration, but AMF assisted the plants in accumulating on average 36% more Fe than non-AMF plants (Cartmill et al., 2007).

Total chlorophyll was not affected by HCO_3^- concentrations $\leq$7.5 mM in non-AMF plants and $\leq$10 mM in AMF plants, probably as a result of the unaffected Fe status at this alkalinity range. Valdez-Aguilar and Reed (2007) estimated a 10% decrease in chlorophyll concentration in vinca plants when irrigation water contained 6.8 mM $NaHCO_3$, which is in close agreement with our observations. In AMF plants there was a steep decrease in chlorophyll concentration when HCO_3^- was increased; this may be due to a dilution effect since at higher levels of HCO_3^- leaf area of AMF plants was higher than that of non-AMF plants.

Phosphorus may form insoluble compounds under high soil pH conditions, which leads to P deficiency in plants. In the present study we report that leaf P concentration was not affected by the increasing pH associated with higher concentrations of HCO_3^-. However, increasing HCO_3^- was associated with a decrease in leaf P content in both AMF and non-AMF plants. However, in AMF-inoculated plants leaf P content was higher than that of non-AMF plants, which may be due to the higher activity of the soluble ALP in AMF-inoculated plants when HCO_3^- concentration was $\leq$7.5 mM.

Reactive oxygen species are byproducts of plant metabolism that are produced in larger quantities under stress conditions (Munné-Bosch and Peñuelas, 2003). These oxygen species can damage cell membranes (Marschner, 1995) if they are not rapidly neutralized by an enzymatic complex and/or other antioxidant compounds (antioxidant system) (Alguacil et al., 2006). In our study, in non-AMF plants there was not a detectable HCO_3^- effect on the activity of the antioxidant system when HCO_3^- concentrations were between 0 and 10 mM, but the activity was markedly decreased when HCO_3^- was at 15 mM. Thus, a very high alkalinity impairs the detoxifying response of plants to reactive oxygen species. However, in AMF plants there was no effect on the antioxidant activity, suggesting that AMF assisted/enhanced the ability of plants to endure the stress imposed by high alkalinity. The more balanced nutrition provided by AMF associations under alkalinity stress may be the cause of the higher antioxidant activity in AMF plants, probably as a result of the higher concentration of Mn, Zn, and Cu, which are required for the function of the antioxidant enzymes (Marschner, 1995).

In terms of leaf nutrient content, inoculation with AMF caused an increase in P, K, Ca, Mg, S, Mn, Zn, Cu, and Mo, when compared to non-AMF plants. Similar results have been reported for a number of species, and they have been attributed to a greater effective root area and penetration of the substrate provided by AMF and to the activation and excretion of enzymes by colonized roots and/or hyphae (Marschner, 1998; Clark and Zeto, 2000), as in the nitrate reductase and soluble phosphatase activities that we are reporting. In the present experiment, the plants were grown in relatively small containers and root growth was not affected, suggesting that the beneficial effect of AMF was due to enhanced nutrient acquisition and transport to the plant, rather than greater effective root area. Even though increasing alkalinity in irrigation water caused a decrease in leaf nutrient content, the decreased trend was mostly quadratic in AMF-inoculated plants, while it was linear in non-AMF plants, suggesting that in AMF plants the decrease under higher alkalinity was less severe than in non-AMF plants.

Kumar et al. (2003) reported a decrease in nitrate reductase activity in leaves of wheat plants irrigated with water of high alkalinity; according to the authors this may be due to a disruption of N metabolism leading to decreased N concentration. However, in alkalinity-tolerant wheat cultivars there was no change in nitrate reductase activity. In this study, the nitrate reductase activity was not affected by HCO_3^-, suggesting that vinca was able to maintain nitrate reduction despite increasing alkalinity. We are reporting the nitrate reductase activity in terms of activity per gram of leaf fresh mass; since leaf DM was less affected by increasing HCO_3^- concentration in AMF-inoculated plants, it is plausible to assume that the reductase activity, on a whole plant basis, was higher in AMF plants exposed at higher alkalinity. This would explain the no effect on leaf N content in AMF plants when HCO_3^- increased up to 10 mM, while in non-AMF plants it exhibited a linear decreasing trend.

5. Conclusions

Vinca is moderately tolerant to HCO_3^--induced alkalinity, but this tolerance was enhanced when plants were inoculated with AMF. Good growth and quality of AMF-inoculated vinca plants can be produced when using irrigation water containing up to 7.5 mM HCO_3^-. The tolerance of AMF-inoculated vinca plants to alkalinity was associated with increased capacity for P uptake due to a higher soluble phosphatase activity at moderate HCO_3^- concentrations, and with the maintenance of other leaf nutrients when HCO_3^- increased up to 10 mM. The antioxidant activity was also enhanced in AMF plants under high alkalinity, suggesting that the plants were able to maintain the detoxifying activity, probably as a result of their enhanced micronutrient status. The nitrate reductase activity was higher in AMF plants exposed to high alkalinity on a whole plant basis, which maintained leaf N content when HCO_3^- increased.

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